Synthesis and Study Thermoreversible Gelation of Biodegradable PLGA-PEG-PLGA Triblock Copolymers in Aqueous Solution

M.Saadatmand, M.J.Abdekhodaie, A.Shojaei
Department of Chemical and Petroleum Engineering, Sharif University of Technology, Tehran, Iran
Saadatmand@che.sharif.ac.ir

Abstract- **A series of triblock copolymers, consisting of a poly(ethylene glycol) centre block joined by two blocks of random lactide/glycolide copolymers, have been synthesized and their characteristics have been determined. Polymer compositions and structures have been assessed via ^{1}H-NMR spectroscopy. The sol(flow)-gel(no flow) was determined by using a test tube inverting method. The results indicated that the length of PLGA block in copolymer is dependent on molar ratio of PEG to (LA+GA) in feed. The balance of hydrophobic and hydrophilic block length of triblock is a critical factor to exhibit a reversible phase transition in water and the molecular weight of PLGA is more significant than the LA/GA ratio of PLGA on the sol-gel transition diagram of triblock. As polymer concentration increases in water, sol-gel transition temperature decreases and gel-sol transition temperature increases.**

Keywords **- Thermoreversible, Triblock, Synthesis, PLGA, PEG, sol-gel, gel-sol**

I. INTRODUCTION

Polymers that display a physicochemical response to stimuli are widely explored as potential drug delivery systems. So far, stimuli studies include chemical substances and changes in temperature, PH and electric field. Homopolymers or copolymers of N-isopropylacrylamide and poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (known as poloxamer) are typical examples of thermosensitive polymers, but they can not be used in drug delivery because they are toxic and non biodegradable[1]. A novel concept, which combines thermogelation, biodegradability and no toxicity, has been proposed for an injectable gel system with better safety and longer gel duration. Jeong and co-workers described different thermosensitive, biodegradable hydrogel based on poly(lactic acid). Block copolymer solutions of PEG and poly (L-lactic acid) were shown to be in the sol state at 45 ^{o}C, and in the gel state at body temperature [2]. However, the need to heat the solution limits the nature of the drugs that can be incorporated in this delivery system, and makes the injection procedure not practical. Later, PEG -b- (lactic acid-co-glycolic acid) -b- PEG (PEG-PLGA-PEG) triblock copolymer solutions were found to form, at room temperature, a free flowing sol that became a transparent gel at 37^{o}C [3]. This polymer exhibited a critical gel concentration of approximately 16 wt% and synthesis of this polymer needs coupling agent, hexamethylene didisocyanate[4].

Low molecular weight PLGA-PEG-PLGA copolymers also exhibit reverse thermal gelation properties at concentration about 10 wt%. The synthesis of these copolymers required no coupling agent, thus they were prepared using a simpler procedure and resulted in more favorable degradation products when applied in vivo than PEG-PLGA-PEG block copolymers. Also the critical gel concentration of these copolymers is lower than PEG-PLGA-PEG block copolymers's[4]. The aqueous solution of PLGA-PEG-PLGA triblock copolymers with a specific composition is a free flowing sol at room temperature and becomes a gel at body temperature. This system avoids using an organic solvent. The temperature-induced sol to gel transition of aqueous solution gives the advantage of easy formulation at the sol state. The drug loading can be achieved by simply mixing the aqueous polymer solution with a drug. The solubility of the drug, even for a hydrophobic drug, can be enhanced by the surfactant nature of the block copolymer in water. The formation of gel starts from the surface of the system by thermal conduction from the body environment, resulting in preventing an initial burst effect. No surgical procedure is necessary to form an implant. The in situ formed gel maintains its integrity for more than 1 month in rats [5]. Therefore, the PLGA-PEG-PLGA system is applicable for injectable long term drug delivery.

In this study, a series of PLGA-PEG-PLGA triblock copolymers with different ratios of LA/GA and PEG/PLGA were synthesized. Phase diagram of these polymers in water were studied. The relationship between gelation and the polymer molecular structure during the temperature-induced transition process, were investigated.

II. EXPERIMENTAL

A.MATERIALS

Lactide was prepared from a 98% lactic-acid solution (Merck) [6]. Glycolide was prepared likewise from glycolic-acid (Merck). Both monomers were purified by multiple recrystalization from ethyl acetate. PEG (Aldrich) (M_n=1000) were purified by dissolution in dried chloroform and then precipitated in n-hexane before use. The catalyst, tin-2-ethylhexanoate (Sigma) and all other chemicals or solvents(Merck) were reagent grade.

B.SYNTHESIS

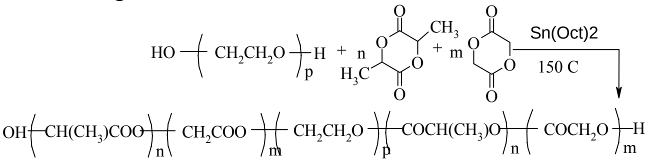
Polyethylene glycol was dried in a two-necked flask under high vacuum and stirring at 150^{o}C for 3hr. Lactide and glycolide were added in the mole ratio of 3:1, respectively, and the reaction mixture was heated under vacuum for 15min. After all the lactide and glycolide were melted, stannous 2-ethyl hexanoate was added and the reaction mixture was further heated at 155^{o}C for 6hr. To remove the unreacted monomers, bath temperature was reduced to 150^{o}C and vacuum was applied. The resultant polymer was dissolved in ice-cold water. After complete dissolution, the polymer solution was heated to effect precipitation of the polymer, and to remove water-soluble low-molecular weight polymer and unreacted monomers. Residual water was removed by freeze drying to give triblock copolymer (PLGA-PEG-PLGA) as a viscous semisolid [7].

C. CHARACTERIZATION

Number average molecular weight and lactide to glycolide ratios were determined by ^{1}HNMR (Bruker 500 MHz) in $CDCl_3$. The sol-gel was determined by using a test tube inverting method with temperature increments of 1°C per each step. Each sample was prepared by dissolving the polymer in distilled water in a vial. The vial containing samples were immersed in a water bath at a constant designed temperature for 10min. Inverting the vial determined a gel state when no fluidity in 1min was visually observed.

III. RESULTS and DISCUSSION

Since the two terminal hydroxyl groups of the PEG essentially have equal reactivities toward monomers, it acts as a macroinitiator for the ring-oppening polymerization of lactones in the side blocks[8]. The following equation shows the general reaction formula:



The FT-IR spectra of the PLGA-PEG-PLGA triblock is presented in figure1.

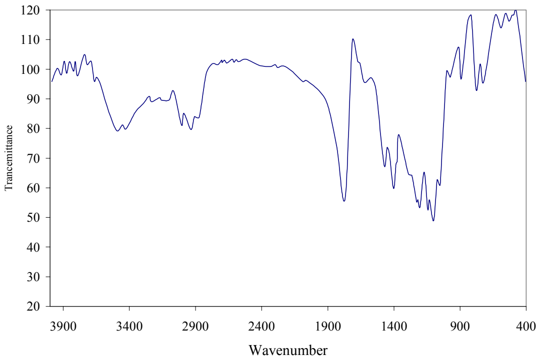


Fig. 1: FT-IR spectra of the PLGA-PEG-PLGA triblock

Two characteristic peaks at 1775cm^{-1} (C=O stretching) and 2883cm^{-1}(CH stretching) on the spectrum demonstrate the PLGA and PEG segments of the copolymers, respectively. The most characteristic absorption of -C-O-C-(ether) stretching at 1150-1085cm^{-1} was overlapped with –CO-O-(ester) stretching.

The ^{1}HNMR spectra of the PLGA-PEG-PLGA triblock is presented in figure2.

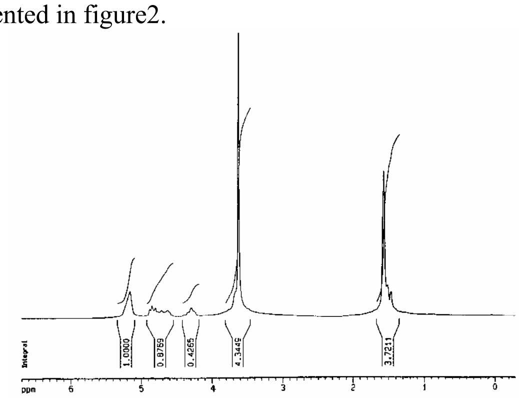


Fig. 2: ^{1}HNMR spectra of the PLGA-PEG-PLGA triblock

The methylene proton of PEG at the linkage between PEG and PLGA appeared at 4.3 ppm and established the synthesis of triblock. The peaks at 5.2, 4.8, 3.6, and 1.55 ppm are the (CH of LA), (CH_2 of GA), (CH_2 of PEG), and (CH_3 of LA), respectively[9]. The following equations were used for calculating the block copolymer molecular weights.

$$p = \frac{M_{nPEG}}{44}$$

$$\frac{4(p-2)+4}{2(n-1)} = \frac{A_{3.6}}{A_{5.2}} \longrightarrow \mathrm{PLA}_n\mathrm{GA}_m\text{-}\mathrm{PEG}_p\text{-}\mathrm{PLA}_n\mathrm{GA}_m$$

$$\frac{4(p-2)+4}{4(m-1)} = \frac{A_{3.6}}{A_{4.8}}$$

$A_{3.6}$, $A_{4.8}$ and $A_{5.2}$ are the peak areas at 3.6, 4.8, and 5.2 ppm, respectively.

With the change of PEG to LA+GA molar ratio in feed, copolymers with different block length of PLGA were synthesized. The molecular weight and compositions of the synthesized block copolymers are summarized in table1.

Table 1: Physical parameters of the synthesized triblock.

Sample	PEG:(LA+GA) (molar ratio) In Feed	Synthesized polymer	M_n^a (g/mol)
1	0.053	$PLA_{18}GA_7$-PEG_{23}-$PLA_{18}GA_7$	1752-1000-1752
2	0.062	$PLA_{17}GA_6$-PEG_{23}-$PLA_{17}GA_6$	1612-1000-1612
3	0.062	$PLA_{16}GA_6$-PEG_{23}-$PLA_{16}GA_6$	1505-1000-1505
4	0.092	$PLA_{12}GA_5$-PEG_{23}-$PLA_{12}GA_5$	1188-1000-1188
5	0.1	PLA_8GA_3-PEG_{23}-PLA_8GA_3	752-1000-752

a) Determined by ^{1}HNMR.

According to the mechanism of reaction mentioned above, PEG acts as a macroinitiator for ring opening polymerization. So the larger the molar ratio of PEG to LA+GA, the more PEG chains is in medium. Therefore, the molecular weight of PLGA block in triblock copolymer decreases. The presented results in table 1 also established this issue [10].

We selected 3 samples of table 1 (presented in table 2) and studied gelation behavior of aqueous solution of them.

Table 2: Physical parameters of the selected triblock.

Sample	M_n^a	LA/GA ^{a} (mol/mol)	PEG/PLGA ^{a} (wt/wt)
1	730-1000-730	75/25	1/1.5
2	1188-1000-1188	75/25	1/2.4
3	1743-1000-1743	73/27	1/3.5

b) Determined by ^{1}HNMR.

The balance of hydrophobic and hydrophilic block length of triblock is a critical factor to exhibit a reversible phase transition in water. Sample3 with PLGA block with a molecular weight higher than 1600 is insoluble in water, and forms hydrogel. Sample1 with PLGA block with a

molecular weight lower than 900 is all soluble in water and can be used as micelles in drug delivery systems. Sample2 show a thermoreversible gelation in water. As presented in figure 3, phase diagram of 26%wt aqueous solution of this triblock has a sol-gel transition at 12^{o}C and a gel-sol transition at 22^{o}C and it precipitates at 35^{o}C.

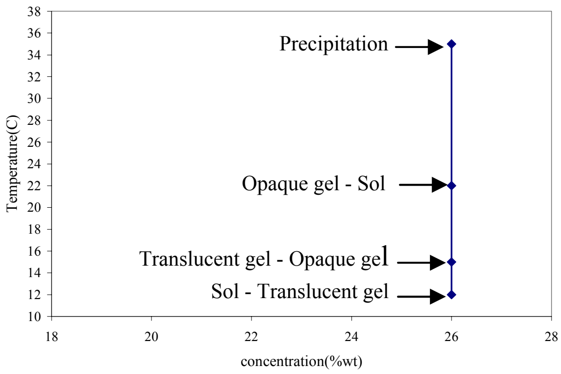


Fig. 3. Phase diagram of 26%wt aqueous solution of sample 2

For study the effect of concentration on phase diagram, two aqueous solution of sample 2 with 26% wt and 20% wt, were prepared and phase diagram of them were obtained. As indicated in figure 4, with increase concentration from 20 to 26%wt, sol-gel transition decreases from 14^{o}C to 12^{o}C and gel-sol transition increased from 19^{o}C to 22^{o}C. As the concentration increases, the interaction between micelles increases, so sol-gel transition changes to lower temperature and gel-sol transition changes to higher temperature.

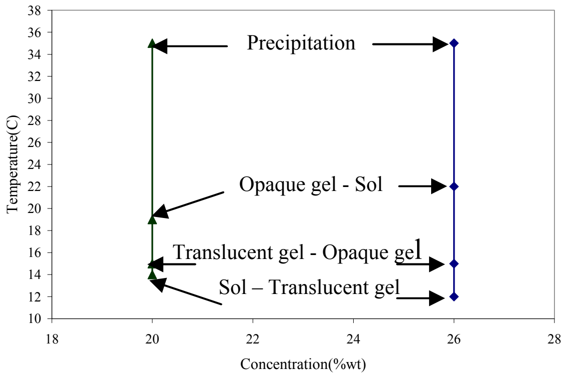


Fig. 4. Effect of aquoues solution concentration on phase diagram

IV. Conclusion

Molar ratio of materials in feed determines the PLGA block length of copolymer. The balance of hydrophobic and hydrophilic block length of PLGA-PEG-PLGA triblock is a critical factor to exhibit a reversible phase transition in water so determining molar ratio in feed is very important. The molecular weight of PLGA is more significant than the LA/GA ratio of PLGA on the sol-gel transition diagram of triblock. As the concentration of polymer in aqueous solution increases, sol-gel transition changes to lower temperature and gel-sol transition changes to higher temperature.

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